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Influence of irrigation strategy and mycorrhizal inoculation on fruit quality in different clones of Tempranillo grown under elevated temperatures

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ABSTRACT

The projected climate scenario for South Mediterranean Europe predicts lower precipitation and higher temperatures that will negatively affect viticulture in the region. The application of moderate deficit irrigation at crucial moments of berry ripening has been found to improve berry quality. Furthermore, grapevine association with arbuscular mycorrhizal fungi (AMF) may improve grapevine's ability to cope with abiotic stresses. Therefore, the aims of this research were: (1) to characterize the response of three clones of *Vitis vinifera* L. cv. Tempranillo to the combination of different water deficit programs and AMF inoculation under elevated temperatures, and (2) to determine whether AMF inoculation can improve berry antioxidant properties under these conditions. The study was carried out on three fruit-bearing cuttings clones of cv. Tempranillo (CL-260, CL-1089 and CL-843) inoculated (+M) or not (−M) with AMF and subjected to two temperature regimes (24/14 °C and 28/18 °C (day/night)) combined with three irrigation regimes during berry ripening. Irrigation treatments were: (i) water deficit from fruit set to veraison (early deficit, ED); (ii) water deficit from veraison to maturity (late deficit, LD); and (iii) full irrigation (FI). Although each Tempranillo clone seemed to have different abilities to respond to elevated temperatures and water supply, in general, at 24/14 °C the LD treatment performed better than ED. Differences among clones were attenuated at 28/18 °C. In addition, potential benefits of the LD treatment were improved by AMF inoculation. Thus, in all clones the loss of anthocyanins at 28/18 °C detected in −M plants after applying LD did not occur in the +M plants. Moreover, AMF inoculation increased must antioxidant capacity in CL-843 under these environmental conditions. Our results suggest that the implementation of measures to promote the association of grapevines with appropriate AMF for each variety could contribute to optimize effects of irrigation strategy on berry properties under future warming conditions.

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1. Introduction

The grapevine is one of the most widely cultivated crops, with a total global surface area of 7.5 million ha under vines. Most harvested grapes are processed into wine, leading to a global production of 274 million hectoliters in 2015, with Spain being the third largest producer in the world (14% of the total world wine production) (OIV, 2016a). However, the future climate scenario for South Mediterranean Europe is not favorable for agriculture in general and for viticulture in particular (Chaves et al., 2010; Lionello et al., 2014) due to the predicted decreased precipitation, increased air and soil temperatures and extreme climate events (IPCC, 2014). Grapevine development has already suffered from significant impact from global climate change (Teixeira et al., 2013). Thus, a growing body of evidence indicates that as the climate warms, grapevine phenology progresses at a faster rate, grapes ripen earlier (Webb et al., 2012), berry sugar content (and subsequent alcohol in the wine) tends to increase (Petrie and Sadras, 2008) and phenolic ripeness is not always achieved (Mori et al., 2007; Sadras and Moran, 2012). In addition, the tendency toward a decreased acidity of must (Sweetman et al., 2014) has potential effects on wine aging capacity.

In the Mediterranean region, the climate may be quite dry during the grapevine growing season and vines may require additional irrigation to counteract water deficit stress (Chaves et al., 2007, 2010). Currently, irrigation of vineyards is below 10% of the total

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area in Europe, but the tendency towards irrigation is increasing in order to mitigate the negative impact of climate change (Costa et al., 2016). Severe water deficit exacerbates the accelerated accumulation of sugars in grapes caused by warm temperatures (Bonada et al., 2015), which results in an imbalance between the levels of sugars and the phenolic ripening in berries (Sadras and Moran, 2012). In contrast, several studies (Santesteban et al., 2011; Zarrouk et al., 2012; Niculcea et al., 2014) have demonstrated that, under moderate water restriction, berries from red wine varieties (such as Tempranillo) had increased levels of sugars and anthocyanins. For several years, moderate deficit irrigation has been applied in order to improve cluster microclimate, increase water use efficiency, control the vegetative development of grapevines, reduce berry size and induce the accumulation of sugars and polyphenols in fruits (Wample and Smithyman, 2002). Different deficit irrigation programs maintain plants at some degree of water deficit for a prescribed part of the season (Basile et al., 2011; Intrigliolo et al., 2012). Nevertheless, high temperatures can constitute a relevant constraint to the implementation and success of the deficit irrigation (Shellie, 2011) and the timing of water deficit might need to be revised to account for the deleterious effects of elevated temperatures in water-stressed plants (Edwards et al., 2011). Thus, it has been reported that alterations in berry primary metabolism (such as sugars, organic acids and amino acids) due to warm temperatures was higher than in secondary metabolism (i.e., anthocyanins and flavonoids), which was mainly affected by timing of water deficit throughout ripening (Torres et al., 2017).

Soil microorganisms can help crops to cope with abiotic stresses (Grover et al., 2011). Amongst these microorganisms, arbuscular mycorrhizal fungi (AMF) have received increasing attention due to their numerous benefits for their host plants. The symbiotic association of plants with AMF is a common phenomenon observed in nearly 80% of plant species, including grapevines (Balestrini et al., 2010). For this reason, considerable progress has been made in the last decade towards the use of these symbiotic fungi to improve grapevine growth and yield. Mycorrhizal symbiosis has been associated with improved growth, increased tolerance against drought and/or enhanced mineral uptake from soils (Trouvelot et al., 2015). Moreover, mycorrhizal plants can accumulate higher levels of phenolic compounds in their tissues than non-mycorrhizal plants and this phenomenon is more evident when plants undergo water deficit rather than optimal irrigation (Baslam and Goicoechea, 2012). In grapevines, mycorrhizal colonization enhances water use efficiency under drought (Valentine et al., 2006) and induces the accumulation of phenolics in leaves (Eftekhari et al., 2012; Torres et al., 2015) and berries (Torres et al., 2016) under optimal irrigation, with these latter results being highly dependent on intravarietal differences of grapevines and air temperatures throughout grapevine cultivation. The phenolic compounds detected in grapes have generated remarkable interest because they have antioxidant properties that are beneficial for human health (Georgiev et al., 2014).

Currently, deficit irrigation in viticulture can be managed in order to increase the concentrations of phenolics in berries with attain the final objective of enhancing must quality and its nutraceutical properties. However, to our knowledge, no studies have assessed the contribution of AMF for improving or maintaining the benefits that different deficit irrigation programs can exert on berry quality in a future scenario of climate change. In our previous studies, we showed that the effects of warm temperatures on berry composition of Tempranillo depended on the deficit irrigation system applied and on the clone chosen (Torres et al., 2017). Moreover, under warm temperatures, the benefits of AMF inoculation on berry properties were also modulated by type of clone (Torres et al., 2016). Taking into account all these precedents, the aims of the current research were (1) to characterize the response of three clones of *Vitis vinifera* L. cv. Tempranillo to the combination of different water deficit programs (pre- and post-veraison deficit irrigation) and AMF inoculation under elevated day/night temperatures; and (2) to determine whether AMF inoculation can improve berry antioxidant properties under different climatic scenarios. Previous research (Antolín et al., 2010; Morales et al., 2016) has demonstrated that fruit-bearing cuttings are a meaningful and useful model system to study the response of berry ripening to environmental factors. Thus, potted vines were used to ensure that all clones experienced the same conditions and to control mycorrhizal inoculation and to have comparable non-inoculated plants.

2. Material and methods

2.1. Biological material and growth conditions

Dormant 400–500 mm long *Vitis vinifera* (L.) cuttings from different clones of Tempranillo were collected during the winter of 2014 from an experimental vineyard of the Institute of Sciences of Vine and Wine (Logroño, Spain) (Denomination of Origin Rioja, North of Spain). Three clones (CL-260, CL-1089 and CL-843) were selected in the field on the basis on their different agronomic traits (Table S1) and on the basis of our previous finding, which showed that phenolic content and antioxidant activity were stimulated by the combination of elevated temperature and AMF inoculation (Torres et al., 2015, 2016). Cuttings of each clone were selected for fruit-bearing according to the steps originally outlined by Mullins (1966) with some modifications as described in Ollat et al. (1998) and Antolín et al. (2010). Briefly, rooting was made in a heat-bed (27 °C) kept in a cool room (4 °C). One month later, the cuttings were planted in 6.5-L plastic pots containing a mixture of vermiculite–sand–light peat (2.5:2.5:1, v:v:v). Properties of the peat (Floragard, Vilassar de Mar, Barcelona, Spain) were pH 5.2–6.0, nitrogen 70–150 mg L^{-1}, P_2O_5 80–180 mg L^{-1}, and K_2O 140–220 mg L^{-1}. The peat was previously sterilised at 100 °C for 1 h on 3 consecutive days. At transplanting, half of the plants were inoculated with the mycorrhizal inoculum GLOMYGEL Vid, Olivo, Frutales (Mycovitro S.L., Pinos Puente, Spain) (+M plants). The concentrated commercial inoculum was derived from an *in-vitro* culture of AMF *Rhizophagus intraradices* (Schenck & Smith) Walker & Schüßler comb. nov. (Krüger et al., 2012) that contained 2000 mycorrhizal propagules (inert pieces of roots colonized by AMF, spores and vegetative mycelium) per mL inoculum. The selection of *in vitro*-produced inoculum of *R. intraradices* was based on two expected benefits: easy application of the product and low colonization of grape roots by contaminant fungi (Vimard et al., 1999). In order to facilitate its application, the concentrated commercial inoculum was diluted with distilled water to a mycorrhizal inoculum of 250 propagules mL^{-1}. Each +M plant received 8 mL diluted mycorrhizal inoculum close to the roots, thus making 2000 propagules in total. In order to compensate for a possible partial disinfection of mycorrhizal spores during the production of high quantities of the commercial inoculum, a filtrate was added to plants that did not receive any inoculum (−M plants) with the objective of restoring the helper microorganisms accompanying spores and hyphae of AMF and which play an important role in the uptake of soil resources as well as on the infectivity and efficiency of AMF isolates (Agnolucci et al., 2015). The filtrate was obtained by passing diluted mycorrhizal inoculum through a layer of 15–20-mm filter paper with particle retention of 2.5 mm (Whatman 42; GE Healthcare, Little Chalfont, UK), and each −M plant received 8 mL filtrate close to the roots.

After transplanting, the fruit-bearing cuttings were transferred to greenhouses, which were adapted to simulate climate change conditions (see details in Morales et al., 2014) until berry matu-

rity was reached. Initial growth conditions were 25/15 °C and 50/90% relative humidity (day/night) regime and natural daylight (photosynthetic photon flux density, PPFD, was on average 850 μmol m^{-2} s^{-1} at midday) supplemented with high-pressure sodium lamps (SON-T Agro Phillips, Eindhoven, Netherlands) to extend the photoperiod up to 15 h and ensure a minimum PPFD of 350 μmol m^{-2} s^{-1} at the level of the inflorescence. Humidity and temperature were controlled using M22W2HT4X transmitters (Rotronic Instrument Corp., Hauppauge, USA). PPFD was monitored with a LI-190SZ quantum sensor (LI-COR, Lincoln, USA). Under these conditions, bud-break took place after one week. Careful control of vegetative growth before flowering improves the partitioning of stored carbon towards the roots and the reproductive structures. Thus, only a single flowering stem was allowed to develop on each plant during growth. Until fruit set, plants were watered twice per day with a nutrient solution (140 mL day^{-1}) with phosphorus level 0.30 mM (Ollat et al., 1998) alternated with water to maintain the soil water content at 80% of pot capacity. The electric conductivity of the nutrient solution adjusted to pH 5.5 was 1.46 ± 0.15 mS cm^{-1} as determined with a conductivity meter (524; Crison Instruments SA, Alella, Spain).

2.2. Experimental design

At fruit set (Eichhorn and Lorenz (E-L) growth stage 27) (Coombe, 1995), which took place one month after bud-break, we established a three-factorial design where two temperature regimes were combined with three water regimes that were applied to plants inoculated (+M) or non-inoculated (−M) with AMF. At fruit set (Eichhorn and Lorenz (E-L) growth stage 27) (Coombe, 1995) fruit-bearing cuttings from +M or −M treatments (36 plants per clone) were exposed to two different temperature regimes: 24/14 °C (day/night) and 28/18 °C (day/night). At this stage, plants have 4–5 fully expanded leaves. The 24/14 °C temperature regime was selected according to average temperatures recorded in La Rioja (1981–2010) (AEMET, Spain) during the growing season. The 28/18 °C temperature regime was selected according to predictions of a rise of 4.0 °C at the end of the present century (IPCC, 2014). Both temperature regimes were maintained until berry ripened (21–23°Brix) (E-L 38 stage). To avoid excessive soil overwarming, which can negatively affect roots, and maintain a stable temperature, pots were shaded by wrapping their lateral surface with a reflecting material (Passioura, 2006). Soil temperature was measured at 5 cm soil depth using temperature probes PT100 (Coreterm, Valencia, Spain) and reached 23 ± 0.5 °C and 28 ± 0.5 °C for 24/14 °C and 28/18 °C air temperature regimes, respectively (Fig. S1).

Within each temperature regime, +M and −M plants from each clone were divided into three groups that were subjected to different irrigation programs. Two deficit irrigation strategies were compared with full irrigation (FI). In the FI treatment, pots were maintained at 80% of pot capacity (volumetric soil water content between 40 and 50%, (m^3 H_2O m^{-3} soil) × 100) from fruit set to harvest. In the water deficit treatments, plants received 50% of the water given to FI plants from fruit set (E-L 27 stage) to onset of veraison (E-L 35 stage) (early deficit, ED) or from onset of veraison (E-L 35 stage, 20–24 fully expanded leaves) to maturity (E-L 38 stage) (late deficit, LD). After ED and before LD, plants were subjected to full irrigation. Volumetric soil water content was monitored with an EC 5 water sensor (Decagon Devices, Inc., Pullman, WA, USA) placed within each pot. Pot capacity was previously assessed by determining water retained after free-draining water had been allowed to pass through the holes in the bottom of the pot. The surface of the plant containers was covered with quartz stones during the experiments to avoid water loss because of evaporation. Water volume supplied to the FI treatment was adjusted to increase plant development according to the daily measurements of the EC 5 water sensor (Fig. S2). Watering was performed with nutrient solution or deionised water in order to supply the different treatments with the same amount of nutrients during water deficit. There were three replicates for each combination of mycorrhizal, temperature, irrigation treatment and clone.

2.3. Mycorrhizal colonization and relative mycorrhizal dependency (RMD)

Root samples were cleared and stained (Koske and Gemma, 1989), and AMF colonization was determined by examining 1-cm root segments (45 per pot) under the microscope. Then, parameters of AMF colonization were calculated for each pot as described previously (Torres et al., 2015). The relative mycorrhizal dependency (RMD) index was estimated as reported by Bagyaraj (1994): RMD = (bunch fresh matter of +M plant) × 100/(bunch fresh matter of −M plant). Determination of RMD allows assessment of the dependency of a crop on the mycorrhizal condition to achieve its maximum growth or yield at a given level of soil fertility. When yield in +M plants is very similar to that achieved in −M plants, the value of RMD is equal to 100%. Values beyond 100% indicate that mycorrhizal symbiosis has improved plant yield. RMD values lower than 100% indicate that mycorrhizal association has reduced yield.

2.4. Plant determinations

Predawn leaf water potential (Ψ_{pd}) was measured with a SKYE SKPM 1400 pressure chamber (Skye Instruments Ltd, Llandrindod, Wales) on three fully expanded leaves per treatment at four stages of berry development: 1) when berries began to soften (Eichhorn and Lorenz(E-L) growth stage 34, green berries) (Coombe, 1995); 2) when berries began to colour and enlarge (E-L 35 stage, veraison); 3) seven days after veraison (E-L 36 stage, veraison + 7); and 4) fourteen days after veraison (E-L 37 stage, veraison + 14). When fruit maturity was reached (E-L 38 stage) plants were harvested separately based on sugar level (21–23°Brix) from berry subsamples (2–3 berries) taken weekly.

The length of phenological phases was recorded as the number of days from fruit set (E-L 27 stage) to veraison (E-L 35 stage), and from veraison (E-L 35 stage) to maturity (E-L38 stage). To obtain yield, bunches were weighed and then 10 berries from each plant were collected and weighed individually. Mean fresh berry mass was determined and berries were separated into skin and flesh. The remaining berries were counted, weighed and frozen at 80 °C for further analysis. The relative skin mass was calculated as the quotient between skin FM and total berry FM expressed as percentage.

2.5. Grape berry determinations

A subsample of 25 berries was crushed and then extracts were centrifuged at 4300*g* at 4 °C for 10 min. The supernatant was used for the following determinations: total soluble solids (mainly sugars) measured with a temperature-compensating refractometer (Zuzi model 315; Auxilab, Beriáin, Spain) and expressed as °Brix; must pH measured with a pH meter (Crison Instruments, Barcelona, Spain) standardised to pH 7.0 and 4.0; titratable acidity measured by titration with NaOH according to International Organisation of Vine and Wine methods (OIV, 2016b) and expressed as g tartaric acid L^{-1}; and L-malic acid measured by an enzymatic method (Enzytec L-Malic Acid; R-Biopharm, Darmstadt, Germany).

Another 25-berry subsample per plant was weighed and taken for the analysis of anthocyanins and total phenols. Anthocyanins were calculated according to the procedure described by Saint-Cricq et al. (1998). The samples of the non-filtered, crushed grape

homogenate were macerated for 4 h at pH 3.2 (tartaric acid). Once maceration was over, the macerated samples were centrifuged at 4300*g* at 4 °C for 10 min. Anthocyanin content was determined in the supernatant by measuring absorbance at 520 nm (Ribéreau-Gayon and Stonestreet, 1965). Total polyphenol index (TPI) was calculated by the absorbance reading at 280 nm in the supernatant obtained after maceration at pH 3.2 (EEC, 1990). All analyses were run in triplicate.

2.6. Antioxidant capacity

Total antioxidant capacity was evaluated on the same must samples used for berry quality determinations by using the free-radical scavenging activity (α, α-diphenyl-β-picrylhydrazyl, DPPH) assay (Brand-Williams et al., 1995). The free radical scavenging activity, using the free radical DPPH•, was evaluated by measuring the variation in absorbance at 515 nm after 30 min of reaction in Parafilm sealed glass cuvettes (to avoid methanol evaporation) at 25 °C. The reaction was started by adding 20 μL of the corresponding sample to the cuvette containing 80 mM (methanol solution) (980 μL) of the free radical (DPPH*). The final volume of the assay was 1 mL. The reaction was followed with a spectrophotometer (Jasco V-630; Analytical Instruments, Easton, MD, USA). The calibration curve was made using gallic acid as a standard.

2.7. Statistical analysis

Statistical analyses were carried out using statistical software the Statistical Package for the Social Sciences (SPSS) (SPSS Inc., Chicago, IL, USA) version 21.0 for Windows. Data were subjected to Kolmogorov-Smirnov normality test due to the small sample size (n = 3). Data appeared to follow a normal distribution and were thus subjected to analysis of variance (ANOVA) within each clone. The test was performed to assess the main effect of the factors temperature (T) (24/14 °C, 24 and 28/18 °C, 28), AMF inoculation (+M and −M) and irrigation program (FI, ED and LD) and the interaction between them. Means ± standard errors (SE) were calculated and when the F ratio was significant ($P \leq 0.05$), a Duncan test was applied. Three-way ANOVA was performed to determine significant differences in all measured parameters. Berry quality parameters and antioxidant activity data were analyzed by principal component analysis (PCA) with the same software to determine general trends in the different samples.

3. Results and discussion

3.1. Mycorrhizal colonization and relative mycorrhizal dependency (RMD)

Under moderate temperatures and optimal irrigation, 100% of plants from CL-260 and CL-1089 had mycorrhizal structures (mainly hyphae and vesicles) (Fig. 1), but percentages of root mycorrhizal colonization did not exceed 10%. In FI plants from CL-843 grown at moderate temperatures, the percentage of root colonization reached 15%, but only 35% of plants showed mycorrhizal structures. Imposition of drought (ED or LD) and/or elevated temperatures (28/18 °C) negatively affected mycorrhizal symbiosis in the three clones of Tempranillo. These low percentages of mycorrhizal colonization achieved at the final stage of fruit ripening could be due to several causes: (1) disinfection of the AMF spores during the production of *in vitro*-mycorrhizal inoculum may have eliminated or at least reduced the bacteria associated with the fungus, thus harming both the survival and the well-timed germination and growth of spores and hyphae included in the applied inoculum (Roesti et al., 2005; Agnolucci et al., 2015); (2) low photosynthetic rates measured at fruit ripening (data not shown) could not allow the production of sufficient carbohydrates to support the mycorrhizal symbiosis; and/or (3) several sinks, such as fruits and mycorrhizal fungus in roots, would be competing for the scarce synthetized photoassimilates during this period of the grapevine life cycle.

In our study, no clear effect of the irrigation regimes and/or air temperatures on the percentage of mycorrhizal colonization was observed. However, several studies have revealed that climate change can affect indirectly (through changes in host plant growth and physiology) and directly the fungal development and the rates of root colonization by AMF (Compant et al., 2010). Although fungal responses to environmental factors can be strain-dependent, in most cases, increased soil temperature favors the mycorrhizal colonization of roots and the spread of hyphal networks while drought reduces AMF colonization. The future scenarios of climate change, however, predict that increased temperature will increase soil dry-

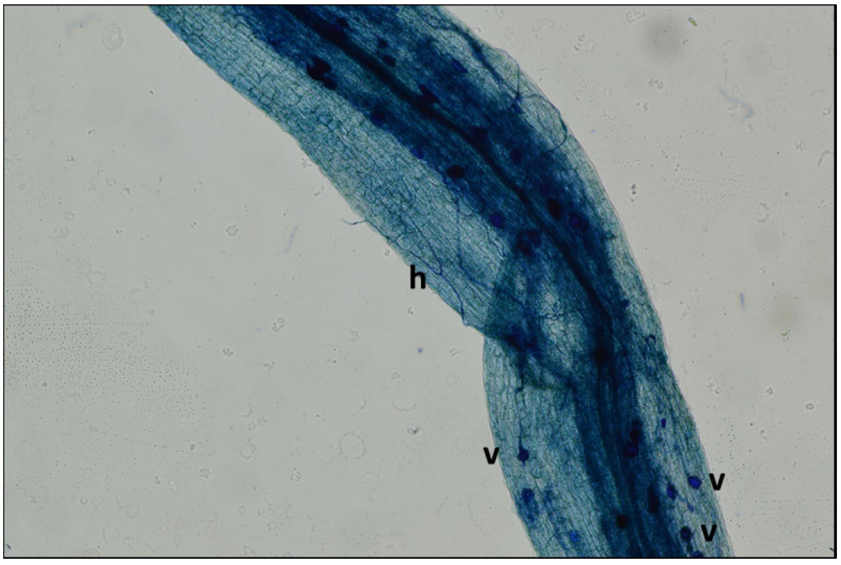

Fig. 1. Microscopic image (×100) of fungal structures from *Rhizophagus intraradices* colonizing roots of inoculated grape plants. h = hyphae; v = vesicle.

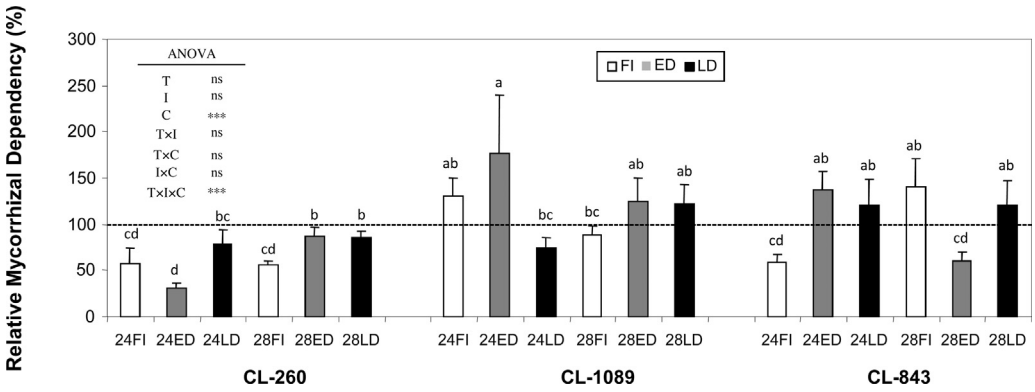

Fig. 2. Relative mycorrhizal dependency (RMD) for yield of fruit-bearing cuttings of Tempranillo clones grown at 24/14 °C or 28/18 °C (day/night) temperatures and subjected to different irrigation regimes (FI, full irrigation; ED, early season deficit irrigation; LD, late season deficit irrigation). Values represent means ± SE (n = 3). Data exceeding 100% indicate that mycorrhizal symbiosis improved yield. A three-way ANOVA analysis was performed to evaluate the effects of temperature (T), irrigation (I), clone (C) and their interaction. ns and *** indicate non-significance or significance at 0.1% probability levels, respectively. Histograms with different letter indicate that values differed significantly ($P \leq 0.05$).

ing, which subsequently will reduce the length of the growing season for mycorrhizal fungal hyphae (Allen et al., 2014).

On the other hand, it is important to note that percentages of mycorrhizal colonization provides information about the root length colonized by AMF but does not indicate the functionality of the symbiosis and thus its benefits for the host plant. A low correlation between the root length colonized by the fungus and the mycorrhizal responsiveness of the host plant means that genetic factors other than those allowing colonization are determining the responsiveness of the host plant to the percent of its root colonized by the mycorrhizal fungus (Parke and Kaeppler, 2000). Calculation of RMD allowed the assessment of the degree to which the studied Tempranillo clones were dependent on the mycorrhizal conditions to produce their maximum yield. Results were highly dependent on the Tempranillo clone and environmental conditions (Fig. 2). In CL-260, mycorrhizal inoculation always decreased fruit yield (RMD values lower than 100%), which may be as a consequence of a sink effect caused by the presence of the fungus in roots that would have increased the translocation of sugars from the aerial part to the roots in detriment to fruit development. The lower yield of +M24-FI plants from CL-260 in comparison with that of −M24-FI was accentuated by the ED treatment, but attenuated when ED was imposed under elevated temperatures(28ED). Pischl and Barber (2016) also found that non-mycorrhizal bell peppers grown at optimal irrigation and ambient temperatures produced more fruits than mycorrhizal plants. According to these authors, under non-stressed conditions the AMF treatments incurred a cost that resulted in reduced growth and fitness relative to uncolonized plants. In agreement with our results, Pischl and Barber (2016) also observed that warm temperatures and drought reduced differences between treatments.

In contrast with findings in CL-260, mycorrhizal association improved yield in plants from CL-1089 grown at 24/14 °C, except for those undergoing LD (Fig. 2). The highest benefit of AMF inoculation on yield was observed when ED was applied. In this case, RMD values almost reached 200%. In CL-843, at 24/14 °C mycorrhizal inoculation enhanced yield in drought stressed plants (24ED and 24LD). However, at 28/18 °C RMD only was significantly improved under full irrigation (28FI). Arbuscular mycorrhizal dependency can strongly vary not only among different plant species (Tawaraya, 2003) but also among cultivars belonging to the same plant species (Tawaraya, 2003; Fahey et al., 2016), which has also been assessed between different grape varieties (Eftekhari et al., 2012; Torres et al., 2015, 2016). Root morphology (root length, length and frequency of the root hairs) seems to be the main factor for determining the mycorrhizal dependency of a given plant species or cultivar because it strongly affects the efficiency of the host plant to acquire resources from the soil (Tawaraya, 2003). Any environmental factor that may alter the root morphology of host plants could also modify their mycorrhizal dependency.

In our study, despite the interclonal differences between CL-1089 and CL-843, in general terms, mycorrhizal inoculation improved yield in plants undergoing adverse conditions (drought, elevated temperatures or both), which reinforces the idea that mycorrhizal symbiosis may be more beneficial for host plants under stressful conditions (Goicoechea et al., 2004) and supports the results indicating that AMF can improve the drought/heat tolerance of vines (Schreiner and Linderman, 2005; Valentine et al., 2006; Schreiner et al., 2007).

3.2. *Phenology and physiological characteristics during berry ripening*

Clones of grapevine cv. Tempranillo tested in this study have significant diversity for some agronomic traits such as length of cycle, yield, bunch mass and berry mass (Table S1). With regard to phenology, vineyard-grown plants of CL-260 and CL-1089 were characterized by a shorter reproductive cycle than CL-843. In our experimental conditions, temperature was the main factor influencing time to reach veraison that was shortened by increasing temperature to 28/18 °C (Table S2). Such observations show a consistent trend towards earlier veraison commonly observed at warm temperatures (Petrie and Sadras, 2008; Webb et al., 2012; Torres et al., 2016). However, in CL-843, at 24/14 °C the ED treatment also contributed to reduce time to reach veraison, as showed by significant interaction between both factors ($T \times I$, $P \leq 0.05$). In the same way, several studies provided support for acceleration of the ripening process due to ED water restriction that was shown by the accelerated onset of anthocyanin synthesis (Castellarin et al., 2007; Herrera and Castellarin, 2016). The hormone abscisic acid (ABA) plays a critical role in regulating berry ripening because berry ABA concentration increases remarkably at veraison, and stimulates anthocyanin synthesis by promoting expression of key biosynthetic genes (Fortes et al., 2015). Under water deficit, it has been reported that ABA pattern accumulation was altered and at veraison, peak of ABA was significantly higher in ED than FI (Niculcea et al., 2014). Possibly, such changes could have contributed to reducing time to reach veraison in the ED treatment. On the other hand, temperature and irrigation were the main factors affecting the time course from veraison to maturity in CL-260 and CL-843. In CL-1089, there was also an additive effect of AMF inoculation, as indicated by the

T	ns	ns	***	***	**	ns	*	***	***	**	ns	***	***	***	***
M	ns	ns	ns	**	**	ns	ns	ns	*	ns	ns	ns	ns	ns	ns
I	***	ns	ns	***	***	***	ns	ns	***	***	***	***	**	***	***
TxM	ns	ns	***	ns	ns	ns	ns	ns	ns	*	ns	ns	ns	*	ns
TxI	ns	ns	*	ns	***	ns	ns	ns	ns	*	*	*	ns	ns	**
MxI	ns	ns	***	***	***	ns	*	ns	ns	ns	ns	ns	ns	ns	*
TxMxI	*	**	***	***	ns	ns	ns	ns	ns	ns	ns	***	ns	**	ns

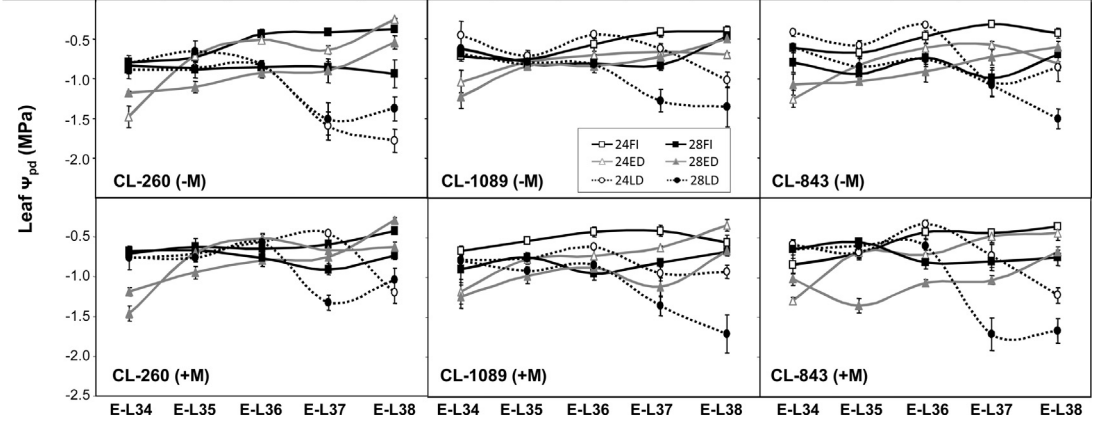

Fig. 3. Pre-dawn leaf water potential (Ψ_{pd}) recorded at different stages of berry ripening in fruit-bearing cuttings of Tempranillo clones inoculated with arbuscular mycorrhizal fungi (+M) or uninoculated (−M), grown at 24/14 °C or 28/18 °C (day/night) temperatures and subjected to different irrigation regimes (FI, full irrigation; ED, early season deficit irrigation; LD, late season deficit irrigation). Values represent means ± SE (n = 3). A three-way ANOVA analysis was performed to evaluate the effects of temperature (T), mycorrhizal inoculation (M), irrigation (I) and their interactions. ns, *, **, and *** indicate non-significance or significance at 5%, 1%, and 0.1% probability levels, respectively.

interaction between temperature, AMF inoculation and irrigation ($T \times M \times I$, $P \leq 0.05$).

Grapevine water status throughout fruit ripening was significantly different per treatments as indicated by the decrease in predawn Ψ_{pd} measured in plants subjected ED or LD compared with FI plants (Fig. 3). During the ED treatment, Ψ_{pd} values reached in CL-260, CL-1089 and CL-843 varied between −1.2 and −1.5 MPa and were not modified by AMF inoculation and/or elevated temperature. In contrast, in the LD treatment, AMF inoculation and elevated temperature modified plant water status. Moreover, significant interactions among factors were observed at stages EL-37 and EL-38 in CL-260 ($T \times I$ and $M \times I$, $P \leq 0.001$), CL-1089 ($T \times M$ and $T \times I$, $P \leq 0.05$) and CL-843 ($T \times I$, $P \leq 0.01$, $M \times I$, $P \leq 0.05$ and $T \times M \times I$, $P \leq 0.01$). Thus, the lowest values of Ψ_{pd} were always recorded in the +M28 treatment and were reached at stage E-L37 (ca. −1.4 MPa) in CL-260 and at stage EL-38 (ca. −1.7 MPa) in CL-1089 and CL-843 (Fig. 3). Similarly, Edwards et al. (2011) showed an additive effect of temperature and water stress on plant water status, which in our study seemed to be more accentuated in +M plants. This low Ψ_{pd} in +M28 treatment could be result from elevated water losses due to high leaf area and/or high transpiration rates. However, this was not the case because leaf area was considerably smaller in +M28 than in +M24 plants (0.80–0.90 m^2 $plant^{-1}$ in +M24 and 0.25–0.40 m^2 $plant^{-1}$ in +M28). Furthermore, +M28 plants had the lowest leaf conductance rates (data not shown). In disagreement with our results, Nikolaou et al. (2003) measured higher Ψ_{pd} in leaves of mycorrhizal 'Cabernet Sauvignon' than in those of non-mycorrhizal plants under drought conditions and the improved water relations in mycorrhizal grapevines were attributed to their higher cytokinin concentrations. However, the exposure of plants to high temperatures can cause reductions in the levels of cytokinins (Todorova et al., 2005; Yang et al., 2016), which may explain the low Ψ_{pd} measured in the +M28 treatment. In addition, it should be noted that although decreases in the tissue Ψ_{pd} depend mostly upon leaf dehydration, it also depend both on initial osmolarity of the cell sap and on cell wall elasticity. Previous studies of our group showed that Ψ_{pd} of mycorrhizal plants involved an increase in the percentage of apoplastic water without a decrease in osmotic Ψ (Goicoechea et al., 1997). This mechanism may allow mycorrhizal plants to tolerate drought without investing solutes into osmotic adjustment, which may be of special importance when the production of carbohydrates to support several sinks (fruits and mycorrhizal fungus) is limited, especially at the end of berry ripening in +M28 plants.

3.3. Berry characteristics

Berry size was mainly modulated by both AMF inoculation and irrigation in CL-260 and CL-1089 (Table 1). Moreover, a significant interaction between irrigation and temperature was highlighted for these clones ($T \times I$, $P \leq 0.01$ and $P \leq 0.05$, respectively), showing that the LD treatment resulted in small berries when plants grew at 28/18 °C. Our findings are in contrast with those presented by Bonada et al. (2015), which showed additive effects (i.e. lack of interaction) between temperature and water for berry mass. In our study, ED was the treatment that most reduced berry mass regardless of temperature and clone, which agrees with previous studies in Tempranillo (Girona et al., 2009; Santesteban et al., 2011; Intrigliolo et al., 2012; Niculcea et al., 2014; Torres et al., 2017). On the other hand, relative skin mass in CL-260 and CL-843 was affected by the three factors applied ($T \times M \times I$, $P \leq 0.001$ for CL-260 and $P \leq 0.01$ for CL-843) (Table 1). In these clones, AMF inoculation and/or elevated temperature accentuated the ability of the ED treatment to increase relative skin mass (Table S3), which could be due to a reduction in berry flesh mass (Roby and Matthews, 2004; Bonada et al., 2015). In CL-1089, temperature had no effect on relative skin mass, with this parameter being mainly modulated by AMF inoculation ($P \leq 0.05$) and irrigation ($P \leq 0.001$). The increase of relative skin mass could be an interesting response under climate change conditions because flavour and colour compounds, which determine wine quality, are located principally in the berry skin. On that basis, at 28/18 °C, berries from 28ED (CL-260 and CL-843) and +M28 (CL-843) treatments will be subject to less dilution of skin compounds during winemaking (Table 1).

Table 1
Main effects and their interactions on berry characteristics from fruit-bearing cuttings of Tempranillo clones (CL-260, CL-1089, CL-843) inoculated with arbuscular mycorrhizal fungi (+M) or uninoculated (−M), grown at 24/14 °C or 28/18 °C (day/night) temperatures and subjected to different irrigation regimes (FI: full irrigation; ED, early season water deficit; LD, late season water deficit).

	CL-260		CL-1089		CL-843	
	Berry mass (g FM berry^{-1})	Relative skin mass (% berry FM)	Berry mass (g FM berry^{-1})	Relative skin mass (% berry FM)	Berry mass (g FM berry^{-1})	Relative skin mass (% berry FM)
Main effects						
Temperature (T)						
24	0.99	30.6	1.00	28.1	0.89	29.5
28	0.86	29.5	0.90	28.1	0.99	28.4
Mycorrhization (M)						
−M	0.99	31.2	0.88 b	30.4 a	0.97	30.0
+M	0.87	28.9	1.03 a	25.8 b	0.91	27.9
Irrigation (I)						
FI	1.16	30.2	1.19	22.9 b	1.09 a	25.8
ED	0.54	36.6	0.47	35.4 a	0.52 b	36.7
LD	1.08	23.3	1.19	26.0 b	1.21 a	24.3
Two-factor interactions						
−M24	1.06	31.4	0.91	30.7	0.90	34.0 a
+M24	0.92	29.7	1.08	25.5	0.88	24.9 b
−M28	0.92	30.9	0.84	30.1	1.04	25.9 b
+M28	0.81	28.1	0.97	26.1	0.94	30.9 a
24FI	1.18 a	34.6 b	1.19 ab	23.1	1.06	28.3 b
24ED	0.51 c	33.2 b	0.46 c	33.9	0.55	33.7 b
24LD	1.29 a	23.8 c	1.35 a	27.3	1.06	26.5 bc
28FI	1.14 a	25.7 c	1.20 ab	22.6	1.12	23.3 c
28ED	0.58 c	40.1 a	0.48 c	37.0	0.50	39.7 a
28LD	0.88 b	22.7 c	1.03 b	24.7	1.36	22.2 c
−M-FI	1.20	34.5	1.10	23.7	1.03	27.3
−M-ED	0.62	35.0	0.47	36.7	0.64	39.3
−M-LD	1.15	24.1	1.06	30.7	1.24	23.3
+M-FI	1.12	25.9	1.29	22.0	1.15	24.3
+M-ED	0.46	38.3	0.47	34.2	0.40	34.1
+M-LD	1.01	22.5	1.32	21.3	1.18	25.3
ANOVA						
Temperature (T)	*	ns	ns	ns	ns	ns
Mycorrhization (M)	*	ns	*	*	ns	ns
Irrigation (I)	***	***	***	***	***	***
T × M	ns	ns	ns	ns	ns	***
T × I	**	**	*	ns	ns	*
M × I	ns	ns	ns	ns	ns	ns
T × M × I	ns	***	ns	ns	ns	**

Values represent means separated by Duncan's test (at P = 0.05). Within columns, means followed by different letters are significantly different as affected by the main factors temperature (24, 28), mycorrhization (+M, −M), irrigation (FI, ED, LD) and their interactions. *P < 0.05; **P < 0.01; ***P < 0.001; ns, not significant (P > 0.05). FM indicates fresh matter.

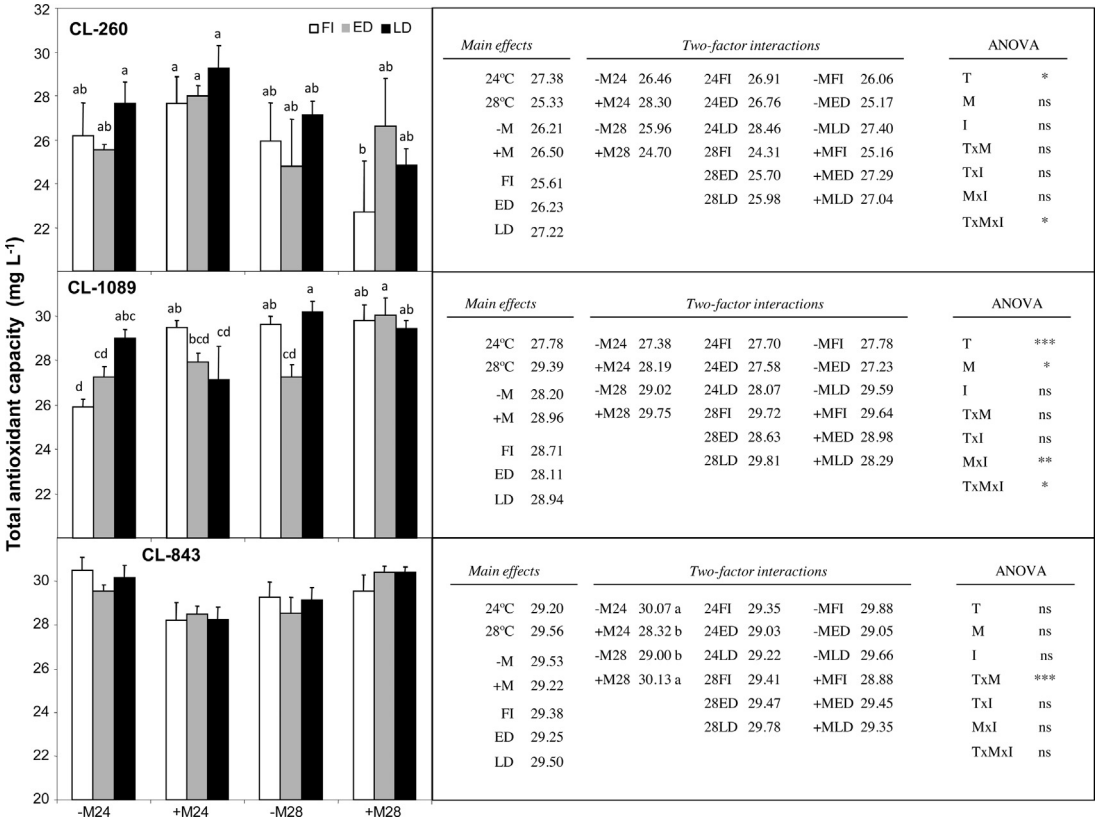

Fig. 4. Total antioxidant capacity of berries from fruit-bearing cuttings of Tempranillo clones inoculated with arbuscular mycorrhizal fungi (+M) or uninoculated (−M), grown at 24/14 °C or 28/18 °C (day/night) temperatures and subjected to different irrigation regimes (FI, full irrigation; ED, early season deficit irrigation; LD, late season deficit irrigation). Values represent means ± SE (n = 3). A three-way ANOVA analysis was performed to evaluate the effects of temperature (T), mycorrhizal inoculation (M), irrigation (I) and their interactions. ns, *, **, and *** indicate non-significance or significance at 5%, 1%, and 0.1% probability levels, respectively. When interaction between the main factors 'temperature, T', 'mycorrhizal inoculation, M' and 'irrigation, I' was significant, histograms with different letter indicate that values differed significantly ($P \leq 0.05$).

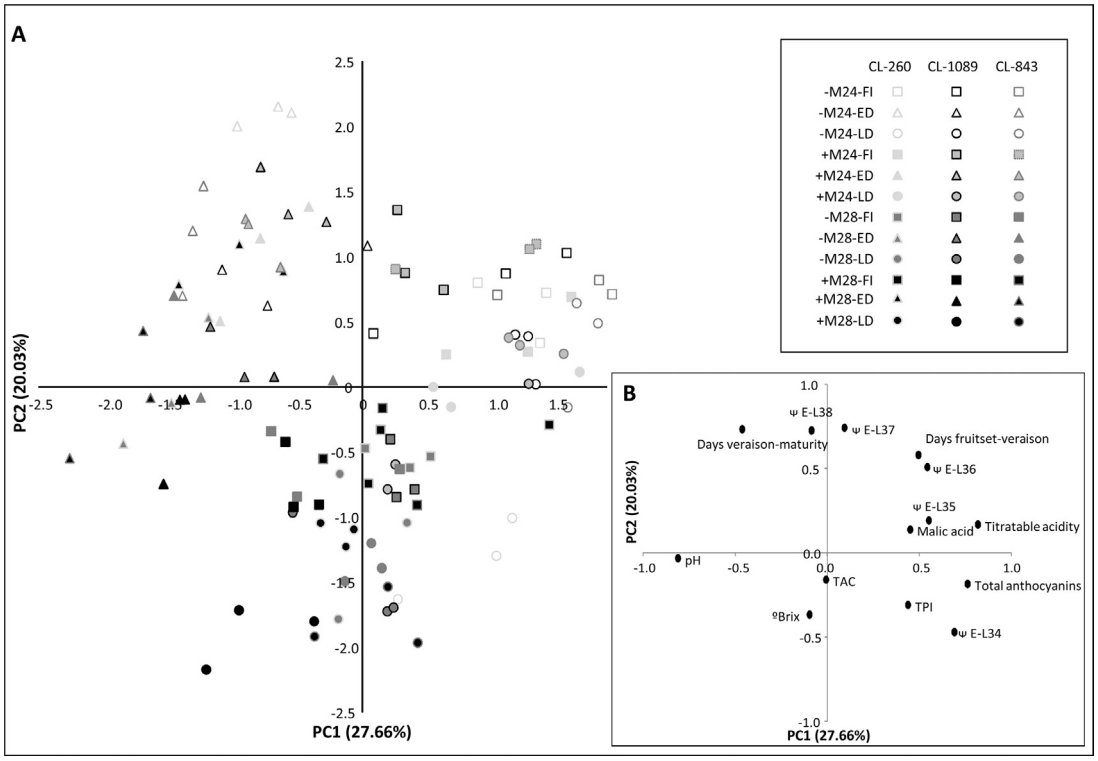

Fig. 5. Principal component analysis score (A) and loading plot (B) obtained from the statistical analysis of plant and berry characteristics and total antioxidant capacity data of 72 studied samples from fruit-bearing cuttings of Tempranillo clones inoculated with arbuscular mycorrhizal fungi (+M) or uninoculated (−M), grown at 24/14 °C or 28/18 °C (day/night) temperatures and subjected to different irrigation regimes (FI, full irrigation; ED, early season deficit irrigation; LD, late season deficit irrigation).

Table 2
Main effects and their interactions on berry quality parameters from fruit-bearing cuttings of Tempranillo CL-260 inoculated with arbuscular mycorrhizal fungi (+M) or uninoculated (-M), grown at 24/14 °C or 28/18 °C (day/night) temperatures and subjected to different irrigation regimes (FI: full irrigation; ED, early season water deficit; LD, late season water deficit).

	Total soluble solids (°Brix)	Must pH	Titratable acidity (g L^{-1})	Malic acid (g L^{-1})	TPI (AU)	Anthocyanins (mg L^{-1})
Main effects						
Temperature (T)						
24	22.0	4.17	5.7 a	7.6	40.0	505.5
28	22.3	4.22	4.6 b	5.6	36.5	456.2
Mycorrhization (M)						
−M	22.0	4.23	5.0	6.6	40.8	484.9
+M	22.4	4.16	5.2	6.5	35.4	473.4
Irrigation (I)						
FI	21.1	3.97 b	5.8 a	6.5	44.4	615.3
ED	22.6	4.46 a	4.1 b	6.5	31.3	341.1
LD	22.8	4.16 b	5.5 a	6.8	41.3	530.9
Two-factor interactions						
−M24	22.0	4.20	5.5	7.1 b	40.5 a	550.9
+M24	22.0	4.10	5.9	8.1 a	39.6 a	467.7
−M28	22.0	4.20	4.6	6.2 b	41.0 a	437.8
+M28	22.7	4.20	4.6	5.0 c	30.9 b	479.7
24FI	21.8 b	4.00	6.3	7.0	48.9 a	713.4 a
24ED	22.4 b	4.50	4.6	8.1	29.0 b	287.9 c
24LD	21.8 b	4.10	6.2	7.8	49.5 a	660.2 a
28FI	20.4 c	3.90	5.4	6.0	41.1 a	541.7 b
28ED	22.8 ab	4.50	3.6	4.9	33.9 b	400.2 b
28LD	23.8 a	4.30	4.8	5.9	35.1 b	433.9 b
−M-FI	20.6	4.10	5.5	7.1 b	51.2 a	630.7
−M-ED	22.7	4.50	4.0	4.7 c	28.4 b	351.6
−M-LD	22.6	4.10	5.6	8.1 a	42.9 ab	472.5
+M-FI	21.6	3.80	6.2	5.9 c	35.5 b	594.9
+M-ED	22.5	4.50	4.2	8.3 a	33.4 b	333.4
+M-LD	23.0	4.20	5.3	5.5 c	39.1 b	608.7
ANOVA						
Temperature (T)	ns	ns	***	***	**	*
Mycorrhization (M)	ns	ns	ns	ns	*	ns
Irrigation (I)	***	***	***	ns	***	***
T × M	ns	ns	ns	**	*	ns
T × I	***	ns	ns	ns	***	***
M × I	ns	ns	ns	***	***	ns
T × M × I	ns	ns	ns	**	ns	*

Values represent means separated by Duncan's test (at P = 0.05). Within columns, means followed by different letters are significantly different as affected by the main factors temperature (24, 28), mycorrhization (+M, −M), irrigation (FI, ED, LD) and their interactions. *P < 0.05; **P < 0.01; ***P < 0.001; ns, not significant (P > 0.05). AU indicates absorbance units.

3.4. Berry quality

Some studies have observed great phenotypic plasticity in berry composition between grapevine varieties in response to warm temperatures (Sadras et al., 2013; Barnuud et al., 2014) and to water deficit irrigation (Basile et al., 2011; Niculcea et al., 2014). Our previous research provided evidence for clonal diversity within Tempranillo that resulted in different abilities to respond to AMF inoculation (Torres et al., 2016) or to the irrigation schedule applied (Torres et al., 2017) under elevated temperatures. Data from the current study showed that interaction between three factors (temperature, irrigation and AMF inoculation) modulated fruit composition to a different extent in each clone. Zarrouk et al. (2016) demonstrated that the interaction between irrigation regime and high temperature controls berry ripening in Tempranillo. Similarly, in CL-260, temperature and irrigation were the main factors modulating berry quality parameters (Table 2). At 28/18 °C, total soluble solids were increased in the water deficit treatments (T × I, P < 0.001), which was explained by the fact that faster accumulation of sugars at warm temperatures may be intensified under water deficit conditions (Bonada et al., 2015). The ED treatment caused the most significant changes in must pH and titratable acidity whereas malic acid was affected by all treatments and growth conditions. This pattern was underlined by a significant interaction among factors (T × M × I, P ≤ 0.01). One of the clearest relationships between temperature and fruit quality occurs with grape berry acidity, whereby high temperatures reduce the concentration and/or increase the breakdown of malic acid. Our data coincide previous studies that showed that warm temperatures produced malate losses in Shiraz (Sweetman et al., 2014), Cabernet Sauvignon and Chardonnay (Barnuud et al., 2014). This has been related to an increased NAD-dependent malic enzyme activity and decreased phosphoenolpyruvate carboxylase and pyruvate kinase activities (Sweetman et al., 2014). Furthermore, total polyphenol index (TPI) and anthocyanin content of CL-260 were also significantly modulated by all factors (Table 2). Thus, TPI tended to be low in response to elevated temperatures, AMF inoculation or ED irrigation, and total anthocyanins decreased in plants grown at 28/18 °C. The decline of anthocyanins under water stress and elevated temperature likely results from the repression of anthocyanin biosynthesis at the onset of ripening and by a high degradation rate at later stages of berry ripening (Bonada et al., 2015; Zarrouk et al., 2016). However, this effect was clearly mitigated in +M28-LD plants, which achieved higher anthocyanins than −M28-LD plants (Table S4). This finding indicates that the beneficial effects produced by AMF inoculation on berry phenolic content under warming conditions (Torres

Table 3
Main effects and their interactions on berry quality parameters from fruit-bearing cuttings of Tempranillo CL-1089 inoculated with arbuscular mycorrhizal fungi (+M) or uninoculated (−M), grown at 24/14 °C or 28/18 °C (day/night) temperatures and subjected to different irrigation regimes (FI: full irrigation; ED, early season water deficit; LD, late season water deficit).

	Total soluble solids (°Brix)	Must pH	Titratable acidity (g L^{-1})	Malic acid (g L^{-1})	TPI (AU)	Anthocyanins (mg L^{-1})
Main effects						
Temperature (T)						
24	21.0	4.15	5.7 a	6.4	36.7	443.8
28	22.6	4.37	3.7 b	4.9	45.1	459.1
Mycorrhization (M)						
−M	20.9	4.23	4.8	6.2	47.4	481.8
+M	22.7	4.30	4.6	5.0	33.9	420.2
Irrigation (I)						
FI	21.8	4.16	4.8	5.6	44.3	508.2
ED	20.4	4.36	4.3	5.2	35.4	285.7
LD	23.1	4.27	5.0	6.1	43.1	586.8
Two-factor interactions						
−M24	19.8	4.10	5.8	6.3 a	39.2 b	490.4
+M24	22.1	4.20	5.7	6.5 a	34.2 b	397.2
−M28	22.0	4.40	3.9	6.2 a	56.6 a	472.4
+M28	23.3	4.40	3.5	3.5 b	33.6 b	445.8
24FI	21.6 a	4.10 c	5.8	5.4 b	34.8 b	584.1 a
24ED	18.6 b	4.30 b	5.2	5.9 b	34.0 b	199.4 c
24LD	22.7 a	4.10 c	6.3	7.9 a	42.1 ab	629.3 a
28FI	22.1 a	4.20 bc	3.9	5.8 b	53.7 a	432.4 b
28ED	22.3 a	4.50 a	3.3	4.4 b	37.3 b	400.8 b
28LD	23.5 a	4.40 a	3.8	4.4 b	44.1 ab	544.3 a
−M-FI	20.6	4.10	5.4	5.6 b	57.9 a	572.0 b
−M-ED	20.1	4.40	4.2	5.6 b	38.1 bc	252.3 c
−M-LD	22.0	4.20	5.0	7.6 a	47.8 ab	659.6 a
+M-FI	23.1	4.20	4.4	5.6 b	30.7 c	444.5 b
+M-ED	20.8	4.40	4.4	4.7 b	32.8 c	319.2 c
+M-LD	24.2	4.30	5.1	4.6 b	38.5 bc	513.9 b
ANOVA						
Temperature (T)	***	***	***	***	***	ns
Mycorrhization (M)	***	*	ns	***	***	*
Irrigation (I)	***	***	ns	ns	**	***
T × M	ns	ns	ns	***	***	ns
T × I	**	*	ns	***	**	***
M × I	ns	ns	ns	**	***	*
T × M × I	ns	ns	ns	*	ns	*

Values represent means separated by Duncan's test (at P = 0.05). Within columns, means followed by different letters are significantly different as affected by the main factors temperature (24, 28), mycorrhization (+M, −M), irrigation (FI, ED, LD) and their interactions. *P < 0.05; **P < 0.01; ***P < 0.001; ns, not significant (P > 0.05). AU indicates absorbance units.

et al., 2016) was higher under LD in comparison to ED, obtaining wines with improved nutritional and nutraceutical value (Gabriele et al., 2016). Similarly, Baslam and Goicoechea (2012) reported that AMF colonization improved the accumulation of anthocyanins in leaves of lettuce especially under water deficit conditions, which may be due to ability of AMF to stimulate the expression of key genes of the phenylpropanoid pathway (Bruisson et al., 2016).

In CL-1089, total soluble solids, must pH and malic acid were modulated by the three factors and/or their interactions, but titratable acidity was only affected by elevated temperatures (Table 3). At 24/14 °C, total soluble solids were reduced by the ED treatment, but such differences disappeared at 28/18 °C (T × I, $P \leq 0.01$). Malic acid decreased in response to elevated temperatures and AMF inoculation under ED and LD, which underlines the interactive effects of all factors on berry quality (T × M × I, $P \leq 0.05$) (Bonada et al., 2015). Furthermore, TPI was significantly modified by the combination of different factors (T × M, $P \leq 0.01$, T × I, $P \leq 0.05$, and M × I, $P \leq 0.01$) (Table 3). In general, TPI diminished in response to AMF inoculation or ED irrigation, but interestingly, the deleterious effect of the −M-ED treatment did not appear in +M-ED plants. Furthermore, total anthocyanin content was significantly modulated by the interaction between all factors (T × M × I, $P \leq 0.05$). At 24/14 °C, AMF inoculation and/or the ED treatment reduced the content of anthocyanins in berries. However, such differences were alleviated at warm temperatures (Table 3) which is in agreement with our previous observations showing that CL-1089 was quite tolerant of elevated temperatures (Torres et al., 2016). Interestingly, although in the study of Torres et al. (2016) AMF colonization deteriorated some aspects of berry quality of CL-1089, the current study shows that the irrigation schedule may modify this pattern. Thus, the loss of anthocyanins detected in −M28-LD plants in comparison with −M24-LD plants did not occur in +M-LD plants when cultivated at 28/18 °C instead of 24/14 °C. This observation highlights the potential benefit of AMF inoculation on phenolic content maintenance under LD conditions applied to CL-1089 (Table S5).

In CL-843, the berry quality parameters were significantly modified by warm temperatures and ED irrigation, as indicated by increased concentration of total soluble sugars, must pH and decreased titratable acidity (T × I, $P \leq 0.001$, $P \leq 0.01$, and $P \leq 0.01$, respectively) (Table 4). In contrast to other clones, the content of malic acid was modulated by mycorrhization and irrigation but not by temperature, which highlights a clonal diversity within the Tempranillo variety in malate metabolism under warm temperatures. Similarly, Sadras et al. (2013) observed great phenotypic plasticity in the responsiveness for some traits such as titratable acidity and must pH. Furthermore, all factors contributed to changes in

Table 4
Main effects and their interactions on berry quality parameters from fruit-bearing cuttings of Tempranillo CL-843 inoculated with arbuscular mycorrhizal fungi (+M) or uninoculated (-M), grown at 24/14 °C or 28/18 °C (day/night) temperatures and subjected to different irrigation regimes (FI: full irrigation; ED, early season water deficit; LD, late season water deficit).

	Total soluble solids (°Brix)	Must pH	Titratable acidity (g L^{-1})	Malic acid (g L^{-1})	TPI (AU)	Anthocyanins (mg L^{-1})
Main effects						
Temperature (T)						
24	20.8	4.13	5.3	6.2	39.0	569.9
28	21.6	4.35	4.0	5.9	40.9	427.0
Mycorrhization (M)						
−M	21.2	4.25	4.6	6.9 a	40.6	509.2
+M	21.2	4.23	4.7	5.2 b	39.2	483.3
Irrigation (I)						
FI	21.5	4.13	5.0	6.1 b	49.0	619.4
ED	20.7	4.43	3.7	5.1 c	32.6	316.1
LD	21.4	4.16	5.3	7.0 a	40.0	599.3
Two-factor interactions						
−M24	19.94 c	4.10	5.2	6.8	36.8 b	581.1
+M24	21.61 ab	4.10	5.3	5.7	41.4 a	557.4
−M28	22.41 a	4.40	4.0	7.1	44.5 a	437.3
+M28	20.82 bc	4.30	4.1	4.8	37.2 b	416.7
24FI	21.37 ab	4.00 b	5.7 a	6.2	49.6	784.6 a
24ED	20.77 b	4.40 a	3.8 bc	4.8	30.5	304.6 d
24LD	20.20 b	4.00 b	6.3 a	7.7	38.3	664.6 b
28FI	21.67 ab	4.30 a	4.4 b	6.1	48.5	454.3 c
28ED	20.58 b	4.40 a	3.5 c	5.3	34.5	326.2 d
28LD	22.65 a	4.30 a	4.2 bc	6.4	41.7	534.0 b
−M-FI	21.48	4.20	4.9	6.7	51.1	678.6
−M-ED	20.95	4.40	3.7	6.2	30.6	305.0
−M-LD	21.10	4.20	5.2	7.8	43.4	612.1
+M-FI	21.50	4.10	5.1	5.5	47.0	560.3
+M-ED	20.40	4.50	3.6	3.9	34.8	328.9
+M-LD	21.75	4.10	5.3	6.3	36.6	586.5
ANOVA						
Temperature (T)	***	***	***	ns	ns	***
Mycorrhization (M)	ns	ns	ns	***	ns	ns
Irrigation (I)	*	***	***	***	***	***
T × M	***	ns	ns	ns	*	ns
T × I	***	**	**	ns	ns	***
M × I	ns	ns	ns	ns	ns	ns
T × M × I	ns	ns	ns	ns	**	***

Values represent means separated by Duncan's test (at P = 0.05). Within columns, means followed by different letters are significantly different as affected by the main factors temperature (24, 28), mycorrhization (+M, −M), irrigation (FI, ED, LD) and their interactions. *P < 0.05; **P < 0.01; ***P < 0.001; ns, not significant (P > 0.05). AU indicates absorbance units.

TPI and anthocyanins as highlighted by the interaction between them (T × M × I, $P \leq 0.01$ for TPI and $P \leq 0.001$ for anthocyanins). Regarding TPI, data showed that the +M28-LD treatment performed better than the +M28-ED treatment (Table S6). With regard to anthocyanins accumulated in berries of plants grown at 24/14 °C, both water restriction regimes (ED and LD) resulted in decreased anthocyanin contents in comparison with levels found in FI plants (−M24-FI or +M24-FI), this effect being more pronounced in −M than in +M plants (Table S6). In general, anthocyanin content was reduced under elevated temperatures but the impact was also lower in +M than in −M plants., In fact, in CL-843, the loss of anthocyanins detected in −M28-LD plants was not found in +M28-LD plants, in comparison with −M24-LD and +M24LD respectively. This finding supports the notion that benefits of AMF under elevated temperature on berry composition (Torres et al., 2016) could be accentuated in combination with water deficit.

3.5. Antioxidant capacity

Fig. 4 shows the results obtained from the DPPH assay performed in must in order to test its total antioxidant capacity. In CL-260 and CL-1089, all factors accounted for modifying total antioxidant capacity of berry extracts (T × M × I, $P \leq 0.05$). In CL-260, there were small changes in total antioxidant capacity, and only +M28-FI plants exhibited a significant reduction in this parameter under warm temperatures. However, in CL-1089, AMF inoculation exerted a positive effect on antioxidant capacity, which was especially accentuated in +M28-FI and +M28-ED treatments. In CL-843, total antioxidant capacity was modulated by the interaction between temperature and mycorrhization (T × M, $P \leq 0.001$). Our data also revealed that elevated temperatures reduced total antioxidant capacity in −M28 plants but it was clearly improved in +M28 plants regardless irrigation regime applied (Fig. 4). These findings suggest that in this clone, the combination of AMF inoculation and elevated temperatures exerted an additive effect in improving antioxidant power (Maya and Matsubara, 2013; Torres et al., 2016).

3.6. Principal component analysis

Fig. 5A shows the score plot obtained by PCA where samples of each clone are grouped in the plot of the first and second principal components: PC1 versus PC2. Here, PC1 accounted for about 27.66% of the total variance while PC2 covered 20.30%. Different Tempranillo clones and AMF inoculated or uninoculated plants could not be clearly distinguished (Fig. 5A). However a clear

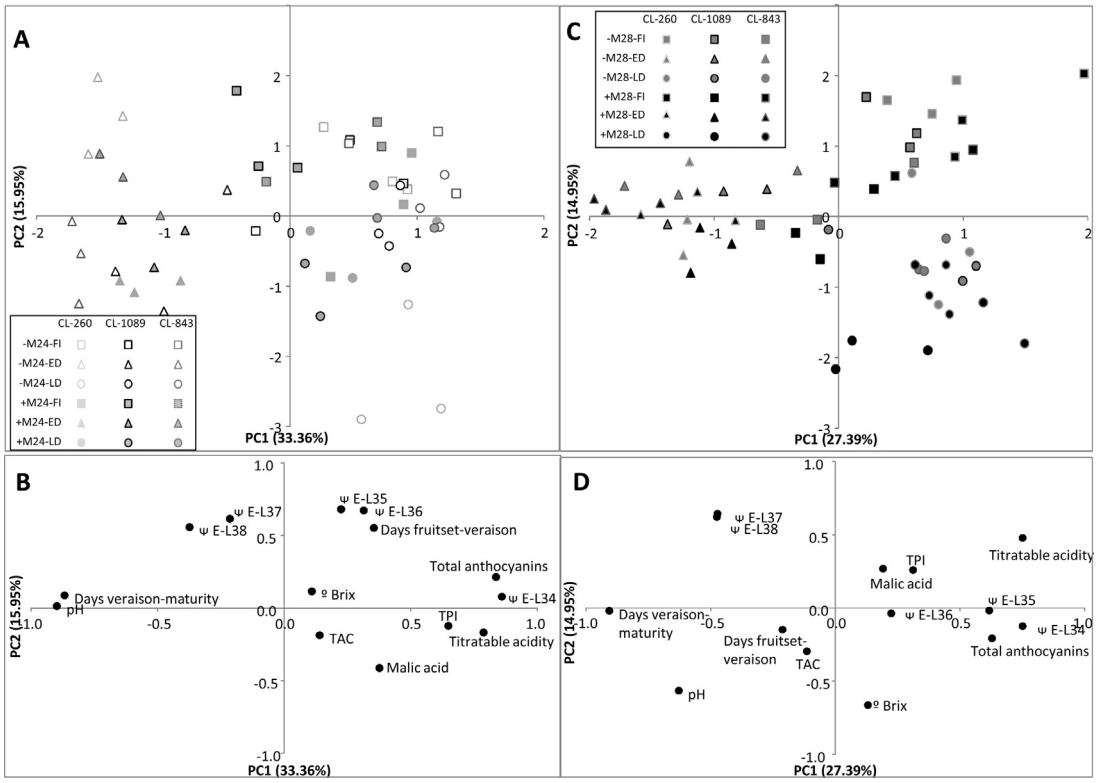

Fig. 6. Principal component analysis score (A, C) and loading plot (B, D) obtained from the statistical analysis of plant and berry characteristics and total antioxidant capacity data of 36 studied samples from fruit-bearing cuttings of Tempranillo clones grown at 24/14 °C (day/night) (A, B) or at 28/18 °C (day/night) (C, D), inoculated with arbuscular mycorrhizal fungi (+M) or uninoculated (−M) and subjected to different irrigation regimes (FI, full irrigation; ED, early season deficit irrigation; LD, late season deficit irrigation).

distinction between plants subjected to different irrigation treatments was shown across PC1. On the other hand, temperature treatments were separated along PC2. These findings reveal that, in our experimental model, abiotic factors (air temperature and water regime) were more determinant than biotic factors (Tempranillo clones and mycorrhization) for berry quality. However, the selection of a given clone of Tempranillo and/or the application of the mycorrhizal inoculum under specific environmental conditions modulated some concrete aspects related to characteristics (Table 1) and quality (Fig. 4, Tables 2–4) of berries, which was demonstrated by the significant interactions between the main factors reflected in the results of ANOVA.

The loading plot (Fig. 5B) highlighted the importance of Ψ_{pd} E-L35, TPI, anthocyanin, pH and acidity levels in explaining variance across PC1. Separation between temperature treatments was related to Ψ_{pd} at the final stages of berry ripening (E-L37 and E-L38), the days between veraison to berry maturity and total soluble solids. This analysis also showed that LD and FI appeared together under both temperature conditions, reinforcing the idea that LD irrigation could continue being valid under a projected warming scenario in order to maintain berry quality and antioxidant properties. With the aim to understand the different performance of biotic factors (clones and mycorrhization) in each temperature two more PCA were conducted (Fig. 6). Fig. 6A and B show the score and the loading plots generated at 24/14 °C while Fig. 6C and D correspond to 28/18 °C conditions. At 24/14 °C irrigation treatments were separated along PC1 which explained the 33.36% of the variance whereas PC2 covered 15.95%. Separation was due to the content of total anthocyanins, TPI, titratable acidity, Ψ_{pd} E-L34, pH, and the number of days between veraison and maturity. Results highlight the similarities between LD and FI at 24/14 °C in the quality parameters and phenology data, but clones and mycorrhizal treatments were not clearly distinguished (Fig. 6A). On the other hand, the two first principal components from data of warming conditions covered 27.39% and 14.95% of the total variance, respectively. ED was distinguished from FI and LD due to PC1, according to their Ψ_{pd} E-L34, Ψ_{pd} E-L35, total anthocyanins, titratable acidity and days from veraison to maturity. However, PC2 allowed establishing a difference between FI and LD treatments (Fig. 6C) in relation with Ψ_{pd} E-L37, Ψ_{pd} E-L38 and the °Brix (Fig. 6D). This result is not surprising due to the treatments were similar till berries reached E-L37 and this period matched with the sugar accumulation period which has been recently shown to be faster under water deficit and warming (Bonada et al., 2015). Additionally, the PC2 conducted at 28/18 °C showed a slightly separation between −M and +M within each irrigation treatment (FI and LD) (Fig. 6D), suggesting that the effect of AMF is more pronounced at 28/18 °C. Under elevated air temperature and LD irrigation the association of Tempranillo grapevine with the mycorrhizal fungus *R. intraradices* favoured the antioxidant capacity of berries (Fig. 4) in CL-843, and induced the accumulation of anthocyanins in berries of CL-260 (Table S4) and CL-843 (Table S6). Because Tempranillo clones responded differently to the inoculation with *R. intraradices* and responses varied according to the environmental conditions, we completely agree with Sinclair et al. (2014) when they concluded that it may be profitable to identify the AMF inoculants most suitable for a given cultivar in a given environment.

4. Conclusions

In a scenario of climate change, effective water management should accomplish the dual purpose of reducing water supply at certain phenological stages to improve grape quality traits while ensuring plant water status to counteract the deleterious effects

of elevated temperature in water-stressed plants. The findings in this study indicate that AMF inoculation may alleviate the negative effects of water restriction and warming conditions in some clones of Tempranillo grapevines. In general, post-veraison (LD) water deficit performed better than pre-veraison (ED) water deficit but such differences were attenuated at elevated temperatures. Furthermore, at 28/18 °C, the potential benefits of LD can be improved by AMF inoculation because the loss of anthocyanins detected in the non-mycorrhizal plants did not occur when plants were inoculated with AMF; this benefit, however, was dependent on Tempranillo intravarietal differences. Our results suggest that the implementation of measures to promote the association of grapevines with AMF could contribute to optimize effects of irrigation strategy on berry properties under future warming conditions. This research also offers the first evidence that the combined effect of water and temperature stress varied depending on AMF inoculation and the clone studied. Future assays with different mycorrhizal inoculants may help to select the most adequate fungal species for benefiting yield and/or berry quality of each clone of Tempranillo cultivated under predicted warming scenarios.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.agwat.2017.12.004.

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